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14. ABSTRACT

120 aminopyrazoles, a new class of c-jun-N-terminal kinase (JNK) inhibitors, have been synthesized and the biochemical IC_{50} has been determined for JNK3, JNK2, JNK1, and p38. In addition, these compounds have been tested in cell-based assays that monitor the inhibition of c-jun phosphorylation and some drug metabolism and pharmacokinetic (DMPK) properties have been measured. The goal of this work is to find JNK3 isoform selective inhibitors. Eight novel aminopyrazoles have been developed with JNK3 selectivity > 20-fold, three novel compounds have been developed with JNK3 selectivity > 50-fold, one novel compound has been developed with JNK3 selectivity > 200-fold, and two compounds have cell-based IC_{50}s < 1 μM. In addition, SR-3306 has been tested for efficacy *in vivo* in transgenic G93A SOD1 mice. Preliminary results show that SR-3306, an aminopyrimidine, is well tolerated with no adverse effects after once daily dosing at 30 mg/kg. Four other aminopyrimidines have been synthesized and are ready for in vitro and in vivo testing to see if they protect motor neuron degeneration.

15. SUBJECT TERMS
ALS; aminopyrazoles; aminopyrimidines; c-jun; DMPK; JNK; SOD1

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INTRODUCTION:

The goal of this project is to test if JNK inhibitors are protective in *in vitro* and *in vivo* models of ALS based on mutations in SOD1. This will be done with JNK inhibitors generated from two different classes of compounds: amino pyrimidines and amino-pyrazoles. The amino pyrimidines are an existing class of compounds that allow for rapid initial *in vivo* analysis and the amino pyrazoles are a newly synthesized class of JNK inhibitors that have yet to be characterized. By utilizing two novel classes of compounds we aim to test if JNK inhibition can be effective in preventing neurodegeneration and motor deficits in ALS animal models.

BODY:

Aim-3: Synthesis, optimization, biochemical, cell biological and DMPK characterization of Amino-pyrazole JNK inhibitors

120 novel amino pyrazoles have been synthesized in the past year and these compounds have been tested in four different biochemical assays (JNK3, JNK2, JNK1, and p38). An HTRF biochemical assay was developed for are four enzymes described. In addition the cell-based potency of these compounds have been tested in SHSY5Y cells. This cell-based assay monitors the inhibition of c-jun phosphorylation in an In-cell Western assay format. Moreover, DMPK assays on select compounds have been executed for solubility, microsomal stability, and inhibition of four different cytochrome P450s.

Tables 1-8 present the HTRF biochemical IC_{50} data for all of the amino pyrazoles synthesized in the first year. The tables present data for JNK3, JNK2, JNK1, and p38. The $IC_{50} \pm SE$ is presented along with the number of replicates (n) for each compound.

Table 9 presents the In-cell Western SHSY5Y cell-based IC50s for all of the compounds tested in cell-based assays. The $IC_{50} \pm SE$ is presented along with the number of replicates (n) for each compound.

Table 10 presents the biochemical IC_{50}, cell-based IC_{50}, microsomal stability, and CYP450 inhibition for eight JNK3 isoform selective compounds that represent the best-in-class aminopyrazole inhibitors.

Table 1. Homogeneous Time Resolved Fluoresence Assay for JNK1, JNK2 and JNK3.
Biochemical IC_{50}s for inhibition of JNK1, JNK2 and JNK3 for novel isoform specific JNK inhibitors. SR-3306 and SP600125 are used as controls for the assay. n; number of experimental repeats. NI; no inhibition, SE; standard error

COMPOUND ID	JNK3			JNK2α1			JNK1α1		
	Mean IC_{50} (nM)	SE	n=	Mean IC_{50} (nM)	SE	n=	Mean IC_{50} (nM)	SE	n=
SP600125	154.1	11.2	n=47	191.9	8.1	n=2	203.9	8.1	n=38
SR-3306	327.0	89.7	n=4	234.4	120.1	n=2	234.4	120.1	n=4
SR-11076	310.5	70.4	n=7	478.5	161.3	n=2	7791.0	1553.2	n=6
SR-11165	42.5	14.8	n=9	144.5	12.4	n=2	2991.3	780.6	n=8
SR-11166	650.7	207.0	n=7	367.4		n=1	21104.5	3470.0	n=7
SR-11367	130.4	37.4	n=6	561.5	178.4	n=2	4544.4	1475.7	n=6
SR-11494	79.9	24.4	n=4	148.5		n=1			
SR-11568	113.8	20.4	n=5	306.4	64.9	n=2	5046.3	1338.9	n=5
SR-11571	83.1	7.3	n=5	159.5		n=1	2941.7	1021.0	n=5
SR-11687	23.0	14.4	n=3	11.9		n=1	679.8	261.9	n=3
SR-11696	138.1	22.8	n=4	181.5	23.7	n=2	6347.0	722.0	n=3
SR-11700	22.7	7.8	n=4	39.4	146.5	n=2	1226.3	119.6	n=4
SR-11948	33.6	5.2	n=4	45.5		n=1	363.7	202.0	n=4
SR-11972	59.8	37.2	n=2	121.1		n=1	2788.0		n=1

Table 2. Homogeneous Time Resolved Fluoresence Assay for p38.
Biochemical IC_{50}s for inhibition of p38 for novel isoform specific JNK inhibitors. Know p38 inhibitor SB203508 was used as controls for the assay. n; number of experimental repeats. N/I; no inhibition

COMPOUND ID	p38	
	Mean IC_{50} (nM)	n=
SB203508	18.9	n=3
SP600125	N/I	n=2
SR-4326	N/I	n=1
SR-11075	N/I	n=1
SR-11076	N/I	n=3
SR-11165	N/I	n=2
SR-11166	N/I	n=2
SR-11173	N/I	n=1
SR-11174	N/I	n=1
SR-11176	N/I	n=2
SR-11248	N/I	n=1
SR-11367	N/I	n=2
SR-11368	N/I	n=1
SR-11369	N/I	n=1
SR-11376	N/I	n=1
SR-11377	N/I	n=1
SR-11494	N/I	n=1
SR-11687	N/I	n=1
SR-11696	N/I	n=1
SR-11700	N/I	n=1
SR-11946	N/I	n=1
SR-11948	N/I	n=1

Tables 3-8. Homogeneous Time Resolved Fluoresence Assay for JNK1 and JNK3.
Biochemical IC_{50}s for inhibition of JNK1 and JNK3 for novel isoform specific JNK inhibitors. The isoform-specificity of the inhibitors is denoted by JNK1/JNK3 IC_{50} ratio
SR-3306, SR-4326 and SP600125 are used as controls for the assay. n; number of experimental repeats. N/I; no inhibition, SE; standard error

COMPOUND ID	JNK3		JNK1α1		n=	JNK1/JNK3 IC_{50} ratio
	Mean IC_{50} (nM)	SE	Mean IC_{50} (nM)	SE		
SR-4326	117.1	14.7	2169.2	169.2	n=30	18.5
SP600125	154.1	11.2	94.8	6.8	n=50	1.6
SR-3306	327.0	89.7	234.4	120.1	n=4	1.4
SR-10946	N/I		N/I		n=2	
SR-10947	N/I		N/I		n=2	
SR-10948	N/I		N/I		n=2	
SR-10949	N/I		N/I		n=2	
SR-10951	N/I		N/I		n=2	
SR-10952	N/I		N/I		n=2	
SR-10953	N/I		N/I		n=2	
SR-10954	N/I		N/I		n=2	
SR-11011	N/I		N/I		n=2	
SR-11012	N/I		N/I		n=2	
SR-11013	N/I		N/I		n=2	
SR-11014	N/I		N/I		n=2	
SR-11015	N/I		N/I		n=2	
SR-11016	7251.5		8804.0		n=1	1.2
SR-11071	N/I		N/I		n=2	
SR-11075	38.4	7.8	169.5	26.6	n=6	4.4
SR-11076	310.5	70.4	7791.0	1553.2	n=6	25.1
SR-11165	42.5	14.8	2715.5	345.0	n=9	63.9
SR-11166	650.7	207.0	21104.5	3470.0	n=5	32.4
SR-11173	49.3	12.1	900.3	344.7	n=4	18.3
SR-11174	225.4	15.4	3384.0	1728.0	n=2	15.0
SR-11176 OLD	469.7	315.7	N/I	N/I	n=3	
SR-11176 NEW	663.9		N/I	N/I	n=1	
SR-11248	130.6	56.2	1938.2	939.3	n=3	14.8
SR-11249	70.7	44.2	179.9	0.7	n=2	2.5
SR-11291	96.5	44.1	803.8	388.3	n=2	8.3
SR-11292	62.8	44.1	536.9	128.3	n=2	8.6
SR-11367	130.4	37.4	4544.4	1475.7	n=7	34.8
SR-11368	73.0	25.0	207.7	93.0	n=3	2.8
SR-11369	938.5	190.9	8088.5	5512.6	n=3	8.6
SR-11375	N/I	N/I	N/I	N/I	n=1	
SR-11376	141.4	44.5	2689.3	558.0	n=2	19.0
SR-11377	5184.5	4049.5	N/I	N/I	n=3	

Table 4.

COMPOUND ID	JNK3		JNK1α1			JNK1/JNK3 IC_{50} ratio
	Mean IC_{50} (nM)	SE	Mean IC_{50} (nM)	SE		
14A	N/I	N/I	N/I		n=1	
F-111	5478.0		1263.0		n=1	
F-112	3623.0		1436.0		n=1	
F-113	871.6	45.0	225.0	45.7	n=2	
F-114	1487.0	254.0	407.7	4.5	n=2	
SR-11404	N/I	N/I	N/I	N/I	n=1	
SR-11406	46.4	4.8	688.1	234.3	n=2	14.8
SR-11407	N/I	N/I	N/I	N/I	n=1	
SR-11475	140.6	61.5	1027.6	389.4	n=2	7.3
SR-11493	50.9	11.0	266.6	81.4	n=2	5.2
SR-11494	79.9	24.4	2368.7	508.9	n=4	29.6
SR-11495	N/I	N/I	N/I	N/I	n=2	
SR-11496	N/I	N/I	N/I	N/I	n=2	
SR-11498	N/I	N/I	N/I	N/I	n=2	
SR-11522	3063.0		12154.0		n=1	4.0
SR-11523	229.6	28.6	3690.5	183.5	n=2	16.1
SR-11524	4588.0	680.0	15254.5	2812.5	n=2	3.3
SR-11568	113.8	20.4	5046.3	1338.9	n=5	44.3
SR-11569	132.5	30.8	2057.7	563.5	n=3	15.5
SR-11570	594.1	222.4	5385.3	512.8	n=3	9.1
SR-11571	83.1	7.3	2941.7	1021.0	n=3	35.4
SR-11572	N/I		N/I	N/I	n=1	
SR-11583	N/I		2927.5	1013.3	n=1	
SR-11584	N/I		115.7	28.1	n=1	
SR-11585	N/I	0.6	3874.0	N/I	n=1	
SR-11589	N/I	0.6	6381.6	0.0	n=1	
SR-11591	N/I		2865.0	N/I	n=1	
SR-11593	416.8		4152.0		n=1	10.0
SR-11598	2472.0		N/I		n=1	

Table 5.

COMPOUND ID	JNK3		JNK1α1		n=	JNK1/JNK3 IC_{50} ratio
	Mean IC_{50} (nM)	SE	Mean IC_{50} (nM)	SE		
SR-11600	N/I		N/I		n=1	
SR-11601	N/I		N/I		n=1	
SR-11687	23.0	14.4	679.8	261.9	n=3	29.6
SR-11688	116.1		1284.0		n=1	11.1
SR-11689	18.0	9.6	494.8		n=2	27.6
SR-11690	434.8		3844.0		n=1	8.8
SR-11691	N/I		N/I		n=1	
SR-11692	N/I		N/I		n=1	
SR-11693	N/I		N/I		n=1	
SR-11694	N/I		N/I		n=1	
SR-11695	162.1		3162.0		n=1	19.5
SR-11696	138.1	22.8	6347.0	722.0	n=4	45.9
SR-11697	188.2	16.6	1837.0	403.0	n=2	9.8
SR-11699	34.0	19.4	757.7	226.5	n=2	22.3
SR-11700	22.7	7.8	1226.3	119.6	n=4	54.0
SR-11701	2561.0		N/I		n=1	
SR-11705	143.5	69.1	1524.8	927.2	n=2	10.6
SR-11739	N/I		N/I		n=1	
SR-11740	63.2		117.1		n=1	1.9

Table 6.

COMPOUND ID	JNK3	JNK1α1	
	Mean IC_{50} (nM)	Mean IC_{50} (nM)	n=
SR-11741	N/I	N/I	n=1
SR-11742	9450.0	N/I	n=1
SR-11743	3544.0	N/I	n=1
SR-11784	N/I	N/I	n=1
SR-11785	10012.0	N/I	n=1
SR-11786	N/I	N/I	n=1
SR-11787	N/I	N/I	n=1
SR-11829	N/I	N/I	n=1
SR-11830	N/I	N/I	n=1
SR-11831	N/I	N/I	n=1
SR-11832	N/I	N/I	n=1
SR-11833	N/I	N/I	n=1
SR-11834	N/I	N/I	n=1
SR-11835	N/I	N/I	n=1
SR-11836	N/I	N/I	n=1
SR-11837	N/I	N/I	n=1
SR-11838	N/I	N/I	n=1
SR-11839	N/I	N/I	n=1
SR-11840	N/I	N/I	n=1
SR-11875	N/I	N/I	n=1
SR-11876	N/I	N/I	n=1
SR-11879	N/I	N/I	n=1
SR-11883	N/I	N/I	n=1
SR-11885	N/I	N/I	n=1
SR-11887	N/I	N/I	n=1
SR-11888	N/I	N/I	n=1
SR-11889	N/I	N/I	n=1
SR-11891	1838.0	8842.0	n=1
SR-11892	N/I	N/I	n=1

Table 7.

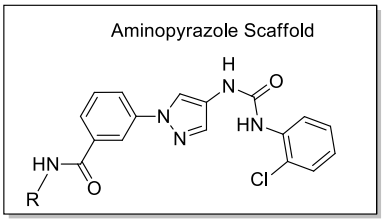
COMPOUND ID	JNK3		JNK1α1			JNK1/JNK3 IC_{50} ratio
	Mean IC_{50} (nM)	SE	Mean IC_{50} (nM)	SE		
SR-11893	N/I		N/I		n=1	
SR-11895	N/I		N/I		n=1	
SR-11896	N/I		N/I		n=1	
SR-11897	N/I		N/I		n=1	
SR-11898	N/I		N/I		n=1	
SR-11932	N/I		N/I		n=1	
SR-11933	279.6	60.6	10729.7	1877.1	n=4	38.4
SR-11934	100.7	16.6	4728.5	1508.3	n=4	47.0
SR-11935	233.4	19.3	18852.3	15501.9	n=3	80.8
SR-11936	743.3	352.7	5550.8	4201.0	n=3	7.5
SR-11946	137.1	56.5	4495.0	1451.7	n=3	32.8
SR-11947	1663.8	1601.6	1232.7	273.0	n=3	0.7
SR-11948	33.6	5.2	363.7	202.0	n=4	10.8
SR-11949	39.9	0.4	3599.9	2673.2	n=2	90.2
SR-11950	3011.5	1855.5	N/I		n=2	
SR-11972	59.8	37.2	2788.0		n=2	46.6
SR-11973	19.1		951.9		n=1	49.7
SR-11974	38.4		1827.0		n=1	47.5
SR-11975	14.7		403.8		n=1	27.5
SR-11976	92.3		5238.0		n=1	56.8
SR-11977	197.9		5032.0		n=1	25.4
SR-11978	33.6		641.6		n=1	19.1
SR-11979	46.3		2229.0		n=1	48.1
SR-11980	6.2		285.5		n=1	46.4
SR-11981	97.8		4270.0		n=1	43.7
SR-11982	45.0		1390.0		n=1	30.9
SR-11983	19.9		777.7		n=1	39.1
SR-11984	8.0		936.7		n=1	116.8
SR-11985	97.8		2775.0		n=1	28.4
SR-3814	96.2		546.8		n=1	5.7
SR-F14A	22.9		11.1		n=1	0.5
SR-F14K	113.4		36.9		n=1	0.3

Table 8.

COMPOUND ID	JNK3	JNK1α1		JNK1/JNK3 IC_{50} ratio
	Mean IC_{50} (nM)	Mean IC_{50} (nM)		
F4B	64.4	24.7	n=1	0.4
F9A	85.5	N/I	n=1	
F9D	124.6	47.7	n=1	0.4
F15A	20.6	N/I	n=1	
F37H	518.4	254.6	n=1	0.5
F43B	234.5	166.8	n=1	0.7
F43C	817.5	621.4	n=1	0.8
F30J	N/I	4178.0	n=1	
48S	94.1	92.7	n=1	1.0
48X	350.2	356.2	n=1	1.0
F3A	26.3	33.0	n=1	1.3
F10D	283.0	37.5	n=1	0.1
F10F	1027.0	191.4	n=1	0.2
F14L	47.1	26.8	n=1	0.6
F37A	5930.0	1838.0	n=1	0.3
F43A	3409.0	614.1	n=1	0.2
SR-11987	20.7	1249.0	n=1	60.2
SR-12052	31.7	3506.0	n=1	110.5
SR-12055	0.1	315.1	n=1	3032.7
SR-12103	181.0	1120.0	n=1	6.2
SR-12127	344.7	>10uM	n=1	
SR-12130	521.8	>10uM	n=1	

Compound ID	Mean Biochemical ± standard error IC_{50} (nM)			In-cell Western IC_{50} (nM) ± standard error Cell lines	
	JNK3	JNK1	JNK1/JNK3 IC_{50} ratio	SHSY5Y IC_{50} nM	
SR-3306	327 ± 89	234 ± 120	1.4 (n=4)	254 ± 60	n=6
SR-11076	265 ± 84	7791 ± 1553	29 (n=5)	905 ± 45	n=4
SR-11165	128 ± 29	2991 ± 780	23 (n=7)	866 ± 204	n=5
SR-11166	195.5	>10,000	29 (n=5)	>10,000	n=4
SR-11367	115 ± 41	4544 ± 1475	39 (n=5)	2331 ± 413	n=3
SR-11376	141 ± 44	2689 ± 558	19 (n=2)	>10,000	n=4
SR-11494	65 ± 27	2368 ± 508	36 (n=3)	1437 ± 243	n=3
SR-11568	113 ± 20	5046 ± 1338	44 (n=5)	3250 ±545	n=5
SR-11571	83 ± 7	2941 ± 1021	35 (n=3)	NI	n=3
SR-11687	23 ± 14	679 ± 261	29 (n=3)	1436 ± 365	n=2
SR-11689	18 ± 9	494	27 (n=2)	1468 ± 261	n=3
SR-11690	434	3844	8.8 (n=1)	NI	n=3
SR-11696	138 ± 22	6347 ± 722	46 (n=4)	9254	n=1
SR-11697	188 ± 16	1837 ± 403	9.8 (n=2)	NI	n=1
SR-11699	34 ± 19	757 ± 226	22 (n=2)	201	n=1
SR-11700	23 ± 8	1226 ± 119	54 (n=4)	>10,000	n=5
SR-11935	233 ± 19	>10,000	(n=3)	3291	n=1
SR-11946	137 ± 56	4495 ± 1508	32 (n=3)	3602 ± 1062	n=2
SR-11947	1663 ± 1601	1232 ± 273	0.7 (n=3)	3333 ± 1120	n=2
SR-11948	20.1 ± 16	278 ± 132	14 (n=3)	>10,000	n=5
SR-11949	39.9 ± 0.4	3599 ± 2673	92 (n=2)	4636 ± 574	n=2
SR-11950	3011 ± 1855	>10,000	(n=2)	>10,000	n=5
SR-9204	55	18	0.3 (n=3)	2147 ± 18	n=2
F9A	85	NI	(n=1)	895	n=1

Table 9. Biochemical and corresponding cell-based IC_{50} of JNK isoform specific inhibitors. In-cell Western assay was performed in neuroblastoma cell line, SHSY5Y. Number of experimental repeats for each compound are denoted by n. NI; no inhibition.



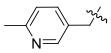
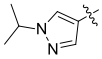
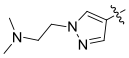
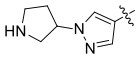
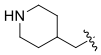
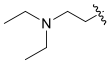
SR#	R	IC50 (nM)[a] JNK3	IC50 (nM)[a] JNK1	AVG IC50 Ratio (JNK1/JNK3)	Cell IC50 (nM)[a]	Mic. stability T1/2 (min)[b] Human	Mic. stability T1/2 (min)[b] Mouse	CYP450 % inh. at 10 μM 1A2 / 2C9 / 2D6 / 3A4
11076		310.5	7791	25.1 (n=6)	905 (n=4)	11.09	16.82	17 / 48 / 83 / 49
11165		42.5	2715.5	63.9 (n=9)	866 (n=5)	31.93	25.63	17 / 60 / 25 / 44
11367		130.	4544.4	34.8 (n=7)	2331 (n=3)	27.96	6.19	10 / 19 / -25 / 26
11568		113.8	5046	44.3(n=3)	3520 (n=5)	39.2	36.86	15 / 49 / 54 / 16
11699		34.0	757.7	22.3(n=3)	201 (n=1)	-	-	-
11055		< 1	315.1	>300 (n=1)	-	-	-	-
11935		233.4	18852.3	80.8 (n=3)	3291 (n=1)	-	-	-
11946		137	4495	32 (n=3)	3602 (n=2)	-	-	-

Table 10: Best-in-class Amino Pyrazoles: Biochemical IC_{50}, cell-based IC_{50}, microsomal stability, and CYP450 inhibition for eight JNK3 isoform selective compounds

Aim-2: Preclinical testing of JNK inhibitors

Hypothesis

In vitro studies performed in the mouse model of familial ALS indicate that the JNK inhibitor SR-3306 completely prevents astrocyte- mediated motor neuron death. As SR-3306 was previously validated and optimized in an *in vivo* model of Parkinson's disease, our objective is to test the beneficial effects of this compound in Tg $SOD1^{G93A}$ mice.

Plan: (A) Determine the Maximum Tolerated Dose (MTD) of SR-3306 for prolonged administration by gavage and start treating Tg $SOD1^{G93A}$ mice with the drug or the vehicle from pre-symptomatic stage P50. (B)Perform motor performance tests and clinical assessment studies during disease progression. (C) Perform morphological studies to assess spinal MN loss, reactive

gliosis, numbers of myelinated axons, and the level of denervation. (D) Perform biochemical tests to measure the level of SR3306 and human SOD1 in tissues at end stage.

KEY RESEARCH ACCOMPLISHMENTS:

Considerable progress is being made on the preclinical study of SR-3306 in the Tg $SOD1^{G93A}$ mice. The *in vivo* study is still ongoing and the SR-3306-treated mice are~ 1 month from reaching end stage.

We perform daily gavage in two groups of 20 Tg $SOD1^{G93A}$ mice (6 females, 14 males in each group) with either SR3306 (30 mg/kg) or with vehicle (1% HPMC in distillated water) that started at the presymptomatic stage (P50). Non-transgenic littermate mice (NTg; n=6) receive a similar dosing regimen in order to test for any side effects of the treatment. Body weight is monitored twice a week and no overt adverse effects due to drug administration have been observed so far.

Starting at P60, we perform once a week a battery of motor tests including the inverted grid test (latency to fall from the inverted grid), the vertical pole test (latency to climb down the pole) and the rotarod test (latency to fall, acceleration for 4 to 40 rpm in 300 s).

At the early symptomatic stage (P109), 6 mice from each Tg SOD1G93A group (treated with either SR3306 or vehicle) and 6 NTg littermate mice were sacrificed. Mice were first anesthetized and Compound Muscle Amplitude Potential (CMAP) was recorded in the right tibialis anterior and gastrocnemius muscles. The tail was then cut to check the genotype and the transgene copy number for which we have a validated RT-PCR protocol. An intracardiac blood draw was performed for hematology and serum chemistry. Finally, mice were perfused and the major organs (heart, spleen, intestines, kidney, and liver) were collected for clinical pathology. Brain and spinal cord and L4 ventral root were harvested for detailed histopathology studies that are ongoing. The left tibialis anterior and gastrocnemius muscles were likewise processed for neuromuscular junction studies.

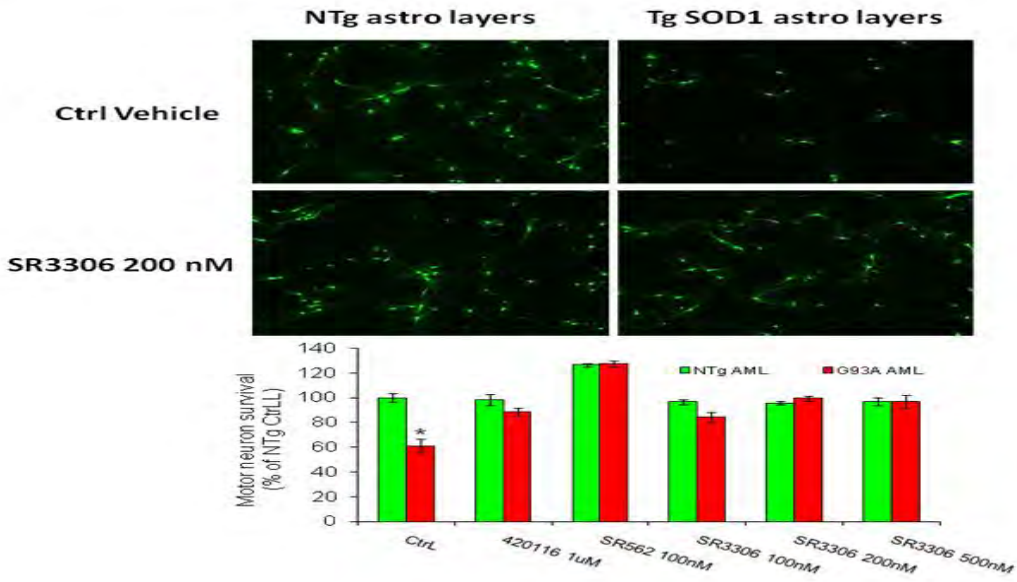
Reportable Outcomes

So far, there are no statistical differences in behavioral scores or in CMAP amplitude between the two groups of Tg $SOD1^{G93A}$ mice that received the JNK inhibitor SR3306 or the vehicle. More in-depth histological studies on muscles and spinal cords need to be completed in order to assess spinal motor neuron numbers and the level of muscle denervation.

Aim 1: Establish the efficacy of a series of amino pyrimidine JNK inhibitors in protecting primary MNs from mutated SOD1-expressing astrocytes.

The efficacy of SR-562 and SR-3306 were tested for protection against mutant G93A SOD1 astrocytes grown with primary motor neurons. Figure 3 presents the protective effect of these two compounds showing that 100 or 200 nM SR-3306 (red bars versus red control bars) was nearly fully protective of the motor neurons.

Fig. 3. JNK inhibitors exert a potent protective effect in our in vitro ALS model



In addition to SR-3306, we also synthesized four other amino pyrimidines that are poised for testing in this *in vitro* assay. These compounds include: SR-2502, SR-3058, SR-3562, and SR-4073. Each of these compounds has been produced in sufficient quantities. These remaining amino pyrimidines will be tested in the *in vitro* motor neuron survival assay in the coming months.

KEY RESEARCH ACCOMPLISMENTS AND REPORTABLE OUTCOMES for entire project: The key research accomplishments from the first year of this project are summarized below.

- > 50g of SR-3306 (an amino pyrimidine) has been synthesized for *in vivo* use

- > 1g SR-3562 (second amino pyrimidine) has been synthesized for *in vivo* use

- Synthesis of three other amino pyrimidines completed (SR-2502, SR-3058, and SR-4073)

- Eight novel amino pyrazoles have been designed with > 20-fold JNK3 selectivity

- Three novel amino pyrazoles have been designed with > 50-fold JNK3 selectivity

- One novel amino pyrazoles have been designed with > 200-fold JNK3 selectivity

- Two novel amino pyrazoles have been designed with < 1 μM cell-based potency

- Good DMPK properties designed into amino pyrazoles class

- assessment of amino pyrimidine JNK inhibitors show efficacy in protecting primary motor neurons *in vitro*

- *in vivo* assessment of one amino pyrimidine nearly completed showing no adverse effects after long term dosing

CONCLUSION: Significant progress has been made in year one of the funding period. First, > 120 novel aminopyrazoles have been synthesized and tested in four different biochemical assays. In addition, many of the potent, selective compounds have been tested in cell-based assays. *In vitro* drug metabolism assays have been tested on a number of compounds helping to define the best compounds in this class for *in vivo* testing for year 2. Secondly, the *in vivo* efficacy model has been established and amino pyrimidine JNK inhibitors have been shown to be safe and well tolerated. Final analysis of efficacy will be established in the next 1-2 months, and testing of amino pyrazoles will commence in year two.

REFERENCES: N/A

APPENDICES: N/A